Control strategies for *Chrysodeixis includens* and *Spodoptera eridania* caterpillars (Lepidoptera: Noctuidae) and selection of resistant cultivars in soybean

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Abstract

*Chrysodeixis includens* and *Spodoptera eridania* caterpillars have been considered the most important pests for *Glycine max* in Brazil. This study evaluated different control strategies for these two important soybean caterpillars and the effects on crop productivity. The control strategies adopted were: BC, IPM, PUI and C (without pest control) and 2 cultivars (BMX Desafio - conventional and TEC Power IPRO – transgenic – Cry 1Ac). The experimental design was in randomized blocks in a factorial scheme (2 x 4) with four replications. The transgenic cultivar TEC Power IPRO presents efficiency in the control of *Chrysodeixis includens* and low efficiency for *Spodoptera eridania*. Harmonious practices such as IPM are the best alternative for soybean crops, to minimize *Chrysodeixis includens* and *Spodoptera eridania* infestations. The control strategy adopted did not influence soybean productivity.

Keywords: Integrated pest management, rational insecticide use, transgenic soybean, plant resistance to insects.

Abbreviations: BC_biological control; IPM_integrated pest management; PUI_prophylactic insecticides use; C_control; PRI_plant resistance to insect; IGRs_insect-growth regulators; DAPE_days after plant emergence; PCLA_percentage of cut leaf area; ANOVA_analysis of variance.

Introduction

The soybean *Glycine max* (L) Merril is one of the most cultivated crops in the world, being used for human and animal food and as a source of renewable energy. However, this crop is damaged by insect pests that cause reduced crop productivity and grain quality (Hoffmann- Campo et al., 2012).

Among the main pests of the soybean are the Lepidoptera: *Chrysodeixis* (*Pseudplusia*) *includens* (Walker), *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae), the caterpillars of the genus *Spodoptera* spp. and soybean stink bugs *Euschistus heros* (Fabricius), *Piezodorus guildinii* (Westwood.) and *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae) which are associated with damage, reduced grain quality and physiological disorders in soybean plants (Chocorosqui and Panizzi, 2004; Temple et al., 2013; Marques et al., 2016).

The main pest control method adopted in soybean cultivation is chemical control using insecticide spraying, which can cause environmental and human health problems as well as select for resistant insect pest populations (Carmo et al., 2010; Martins and Tomquelski, 2015).

In integrated pest management programs (IPM), control strategies need to be employed and the interaction of PRI (conventional or transgenic) needs to be compatible with all control methods (Boiça Junior et al., 2015; Souza et al., 2016). PRI was shown to be efficient, reducing pest populations below the level of economic damage consequently reducing production costs and insecticide use (Smith, 2005; Seifi et al., 2013). However, little is known about the interaction between RPI, insecticide use and biological control in pest control in soybean crops (Zalucki et al., 2009; Bueno et al., 2011).

The development of conventional soybean cultivars resistant to insects, such as IAC-17, IAC-18 and IAC-100, provided greater pest control efficiency (Miranda et al., 2003). However, with the introduction of transgenic soybean plants in 2013, expressing 6-endotoxins (Cry 1 Ac proteins) from the bacterium *Bacillus thuringiensis* Berliner (Bt) in Brazil, new research options for management strategies are necessary as a tool in soybean IPM (Souza et al., 2014a; Souza et al., 2014; Souza et al., 2016). This technology efficiently controls velvet bean caterpillar (*Anticarsia gemmatalis* - Hübner), soybean looper (*Chrysodeixis includens* - Walker and Rachiplusia no-Guénéé), soybean budborer (*Crocidosema aporema* - Walsingham), tobacco budworm (*Helothis virescens* - Fabricius), lesser cornstalk borer (*Elasmopalpus lignosellus* - Zeller), corn earworm (*Helicoverpa zea* - Boddie) and old world bollworm (*Helicoverpa armigera* - Hübner) (Marques et al., 2016).

The evaluation of different pest control strategies is interesting, assisting soybean growers to choose efficient methods in harmony with the environment (Bueno et al., 2010). Bueno et al., 2011 observed different levels of pest infestation depending on the control strategy adopted. In biological control and IPM - insecticide, a higher pest
incidence was observed, however crop productivity remained the same when prophylactic insecticide applications were adopted. It was concluded that excessive insecticide use does not generate an increase in soybean productivity and that IPM practices are the best alternative for pest control.

This study aimed to evaluate different management strategies for *Chrysodeixis includens* and *Spodoptera eridania* caterpillars (Lepidoptera: Noctuidae) in soybean crops.

**Results**

The caterpillar infestation presented a statistical difference between the cultivars at 30 DAPE. The soybean looper *Chrysodeixis includens* (Lepidoptera: Noctuidae) occurred in higher numbers in BMX Desafo (conventional) (*F* = 35.92; *P* ≤ 0.0005) than in TEC Power IPRO (*F* = 6.92; *P* = 0.0201). The control strategies did not differ statistically in this period for *C. includens* (*F* = 0.56; *P* = 0.6447). *Spodoptera eridania* (Lepidoptera: Noctuidae) showed the highest infestation in TEC Power IPRO (*F* = 6.32; *P* = 0.0201) in the IPM strategy, without statistical difference from the BC and C (*F* = 2.95; *P* = 0.0561) (Table 1).

*C. includens* infestations at 45 DAPE were higher in BMX Desafo (*F* = 63.48; *P* ≤ 0.0005) and the control strategies did not differ at this stage (*F* = 1.32; *P* = 0.290). The cultivars and control strategies did not differ statistically for *S. eridania* infestation at 45 DAPE (*F* = 0.63; *P* = 0.430). Defoliation was higher in the cultivar BMX Desafo (*F* = 63.87; *P* ≤ 0.0005), and PUI (*F* = 5.35; *P* = 0.0067) provided the highest percentage of defoliation (Table 1).

At 60 DAPE, the highest *C. includens* infestation was in BMX Desafo (*F* = 81.55; *P* ≤ 0.0005) and the control strategies did not differ (*F* = 1.18; *P* = 0.3338). The cultivars did not differ for *S. eridania* (*F* = 2.68; *P* = 0.1160), and the greatest caterpillar infestation was in BC, however it did not differ from PUI and C (*F* = 2.52; *P* = 0.0856). The percentage of defoliation was higher in BMX Desafo (*F* = 56.16; *P* ≤ 0.0005) and the control strategies did not influence this parameter (*F* = 1.31; *P* = 0.2961) (Table 1).

At 75 DAPE, the *C. includens* infestation was highest in BMX Desafo (*F* = 81.75; *P* ≤ 0.0005). The PUI control strategy (*F* = 3.79, *P* = 0.0255) led to the greatest *C. includens* infestation. Cultivars and control strategies did not influence *S. eridania* infestation at 75 DAPE. The highest percentage of defoliation was observed in BMX Desafo (*F* = 63.82; *P* ≤ 0.0005) in the BC and PUI (*F* = 8.63; *P* = 0.0063) (Table 1).

The interaction of cultivars versus control strategies at 45 DAPE for the percentage of defoliation, showed the highest value in BMX Desafo in C. The control strategies did not influence the percentage of defoliation in TEC Power IPRO. The number of *C. includens* at 75 DAPE was highest in BMX Desafo, while TEC Power IPRO presented no *C. includens* infestations regardless of the control strategy adopted. For the percentage of defoliation at 75 DAPE, the highest values were in BMX Desafo in C and IPM. The control strategies did not influence the percentage of defoliation during this period (Table 2).

The highest productivity was in BMX Desafo compared to TEC Power IPRO (*F* = 18.50; *P* = 0.0053) while the control strategies (*F* = 1.99; *P* = 0.1463) did not influence soybean crop yields (Figure 1).

**Discussion**

In the evaluation of 30 DAPE, low infestation by the two caterpillar species was observed, with a greater number in BMX Desafo (conventional), however without defoliation. The presence of *C. includens* was observed in the TEC Power IPRO (transgenic) cultivar. These caterpillars were neonates that died from feeding on the soybean plant and ingesting Bt protein (Cry 1 Ac) (Bernardi et al., 2014). This protein associates with receptors in the microvilli of the intestine of the insects, causing osmotic lysis and consequently caterpillar death (Bobrowski et al., 2003).

The fact that *S. eridania* fed on the transgenic TEC Power IPRO cultivar shows that the Cry 1 Ac protein is not effective in the control of this caterpillar. *Spodoptera cosmioides* (Lepidoptera: Noctuidae) had completed the biological cycle in transgenic soybean cultivars (Boiça Junior et al., 2015). This protein presents low toxicity to *S. cosmioides*, *S. eridania* and *Spodoptera frugiperda* (Lepidoptera: Noctuidae), being a strategy inadequate to reduce the population of these caterpillars in the soybean crop (Santos et al., 2009; Bernardi et al., 2014). The control of some species of the genus *Spodoptera* in Bt soybean is not yet clear, such as *S. eridania* (Liu et al., 2005; Naranjo, 2005; Faria et al., 2007; Whitehouse et al., 2007); however, in some regions of Brazil, this species has been a key pest in soybean crops (Bueno et al., 2011; Bernardi et al., 2014).

In the evaluations at 45, 60 and 75 DAPE, the effectiveness of Bt protein (Cry 1 Ac) present in TEC Power IPRO was verified in the control of *C. includens*, where infestation of plants by this caterpillar was not observed. This shows the importance of Bt soybean in the IPM of leafhopper caterpillars, reducing the damage caused by these insects and reducing the use of chemical pesticides (Bobrowski et al., 2003).

The presence of *S. eridania* was observed in all evaluations of soybean crops. Other studies have shown the low efficiency in the control of caterpillars of the genus *Spodoptera* with adoption of Bt soybean (Santos et al., 2009; Bernardi et al., 2014). The low efficiency of Cry 1 Ac in the control of caterpillars of the genus *Spodoptera* is possibly related to the tolerance of this group to different types of Bt protein, the genetic variability between the populations of the pest and/or the inactivation of the insecticidal protein by proteases produced by these caterpillars (Miranda et al., 2001; Rahman et al., 2012).

Bt soybean shows low efficiency in the control of species of the genus *Spodoptera*, and other control tactics should be implemented under field conditions. This was exemplified in the observations of the control strategies throughout the evaluations. At 30 DAPE, the lowest *S. eridania* infestation was observed in the BC and C, without differing from the PUI. At 45 DAPE, the lowest *S. cosmioides* infestation was in the TEC Power IPRO cultivar in the CB and IPM, without differing from C. At 60 DAPE, no *S. eridania* infestation was observed in the IPM.

In general, throughout the evaluations, the lowest *S. eridania* infestations were observed in TEC Power IPRO (Cry 1Ac) using the BC and IPM strategies. This demonstrates the importance of adopting harmonious practices such as BC and IPM in soybean cultivation systems. Bueno et al. (2011)
Table 1. Number of *Chrysodeixis includens* (Lepidoptera: Noctuidae) and *Spodoptera eridania* (Lepidoptera: Noctuidae) in one linear meter at 30, 45, 60 and 75 DAPE in different control strategies. Cristalina, Goiás, Brazil. 2016/2017.

<table>
<thead>
<tr>
<th>Cultivars (A)</th>
<th>30 DAPE</th>
<th>45 DAPE</th>
<th>Def.</th>
<th>60 DAPE</th>
<th>75 DAPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.i</td>
<td>S.e</td>
<td>C.i</td>
<td>S.e</td>
<td>Def.</td>
</tr>
<tr>
<td>BMX Desafio</td>
<td>2.62 a</td>
<td>0.25 b</td>
<td>2.87 a</td>
<td>0.12 a</td>
<td>5.62 a</td>
</tr>
<tr>
<td>TEC Power IPRO</td>
<td>0.25 b</td>
<td>0.87 a</td>
<td>0.00 b</td>
<td>0.25 a</td>
<td>0.62 b</td>
</tr>
<tr>
<td>F (A)</td>
<td>35.92 *</td>
<td>6.32 *</td>
<td>63.48 *</td>
<td>0.63 ns</td>
<td>63.87 *</td>
</tr>
<tr>
<td>P value (A)</td>
<td>&lt;0.0005</td>
<td>0.0020</td>
<td>&gt;0.0005</td>
<td>0.4300</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Treatments (B)

| 1 - BC | 1.00 a  | 0.37 ab | 1.50 a  | 0.25 a  | 2.06 b  |
| 2 - IPM | 1.62 a  | 1.12 a  | 1.12 a  | 0.00 a  | 2.12 b  |
| 3 - PUI | 1.50 a  | 0.62 ab | 2.00 a  | 0.13 a  | 5.12 a  |
| 4 - C | 1.62 a  | 0.12 b  | 1.12 a  | 0.37 a  | 3.25 ab |
| F (B) | 0.56 ns | 2.95 *  | 1.32 ns | 1.06 ns | 5.35 ** |
| P value (B) | 0.6447 | 0.0056 | 0.2900 | 0.0067 |
| F (A x B) | 0.96 ns | 0.92 ns | 1.32 ns | 2.75 ns | 4.92 ** |
| P value (A x B) | 0.4200 | 0.4450 | 0.2900 | 0.0090 |

Means followed by the same letter do not differ significantly according to the Tukey test at 5% probability. C.i = *Chrysodeixis includens*; S.e = *Spodoptera eridania*; Def = Percentage of defoliation.

Fig 1. Productivity (kg/ha) in two soybean cultivars (A) using different control strategies (B) infested by *Chrysodeixis includens* and *Spodoptera eridania* (Lepidoptera: Noctuidae). The different letters on the bars represent averages statistically separated by the Tukey test (p <0.05). F treatments (A) = 18.50 ** and P Value (A) = 0.0053; F treatments (b) = 1.99 and P Value (b) = 0.1463. BC = Biological control; IPM = Integrated pest management; PUI = prophylactic insecticide use and C = Control. Cristalina. Goiás. Brazil. 2016/2017.
observed that PUI used in soybean did not result in higher grain yields and that this practice requires large amounts of pesticides, which may damage crop sustainability. Therefore, the use of IPM remains the best alternative for pest management in soybean cultivation. These results corroborate those of Bueno et al. (2011) in which the control strategies adopted in the present experiment did not influence soybean crop productivity.

Materials and Methods

Plant materials and experimental site

Seeds of the BMX Desafio - conventional and TEC Power IPRO - transgenic (Intacta) cultivars were sown under field conditions on 20 Nov. 2016. The experiment was developed in Cristalina, Goiás, Brazil, at the geographical coordinates: Latitude: 16° 46' 07" S and Longitude: 47° 36' 49" W. According to the Köppen classification, the climate of the region is type Cwb, which is defined as a tropical warm and temperate climate with average annual precipitation of 1422 mm, rainy season defined as a tropical warm and temperate climate with average temperature in the region during dur recognized. The climate of the region is type Cwb, which is defined as a tropical warm and temperate climate with average annual precipitation of 1422 mm, rainy season defined as a tropical warm and temperate climate with average temperature in the region during the summer and a dry season in the winter.

Field plots and trails

The experimental design was randomized blocks in a factorial scheme with four replications. The first factor was 2 cultivars (BMX Desafio - conventional and TEC Power IPRO – transgenic – Cry 1Ac) and the second factor consisted of the strategies: BC, IPM, PUI and C (without pest control). The design consisted of 4 linear plots, with 0.5 m spacing between the rows with a density of 20 plants/linear meter. The four central rows were considered the usable area.

In CB, nucleopolyhedrovirus (VPN-HsSNPV) was used. For the IPM, insecticides from the IGRs group were used, which are considered selective for natural enemies. For the PUI, insecticides from the pyrethroid group were used, which are normally preferred by soybean farmers in Brazil. They are usually cheaper and mixed with herbicides and fungicides and sprayed in a single operation (Bueno et al., 2011). The applications of herbicide and fungicide were made when necessary. All products were sprayed with a CO2 pressurized backpack sprayer (Hericat®) set for a spray volume of 150 L/ha using a Micron 1100B2 application tip. The cultural treatments were carried out according to Sediyama, 2009, except the insecticide application.

Pest sampling

The caterpillars were sampled during the vegetative and reproductive stages (Fehr and Caviness, 1977) using the sampling cloth methodology (Hoffmann-Campo et al., 2012). Sampling started at 30, 45, 60 and 75 DAPE counting the number of caterpillars in one linear meter per plot. The percentage of defoliation was estimated visually (0 - 100%), attributing a PCLA representative of all plots (Pinheiro et al., 2005; Lourenção et al., 2010). When the plants reached physiological maturity, 4 m of the two central rows were harvested to determine grain productivity. The weight and moisture content of each sample were corrected to 13% seed moisture to obtain the productivity.

Statistical analysis

The data was submitted to ANOVA using the Shapiro-Wilk test to evaluate the normality of the residues. The averages were compared using the Tukey test at 5% probability. All analyses were performed using R software version 3.2.2 (R Core Team, 2017).

Conclusion

The transgenic TEC Power IPRO (Cry 1 Ac) cultivar presents efficiency in the control of Chrysodeixis includens and low efficiency for Spodoptera eridania. Harmonious practices such as IPM are the best alternative for pest management in soybean cultivation.
alternative in soybean crop systems to minimize C. includens and S. eridania infestations. The control strategies adopted did not influence soybean crop productivity.

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